FIGURE 1

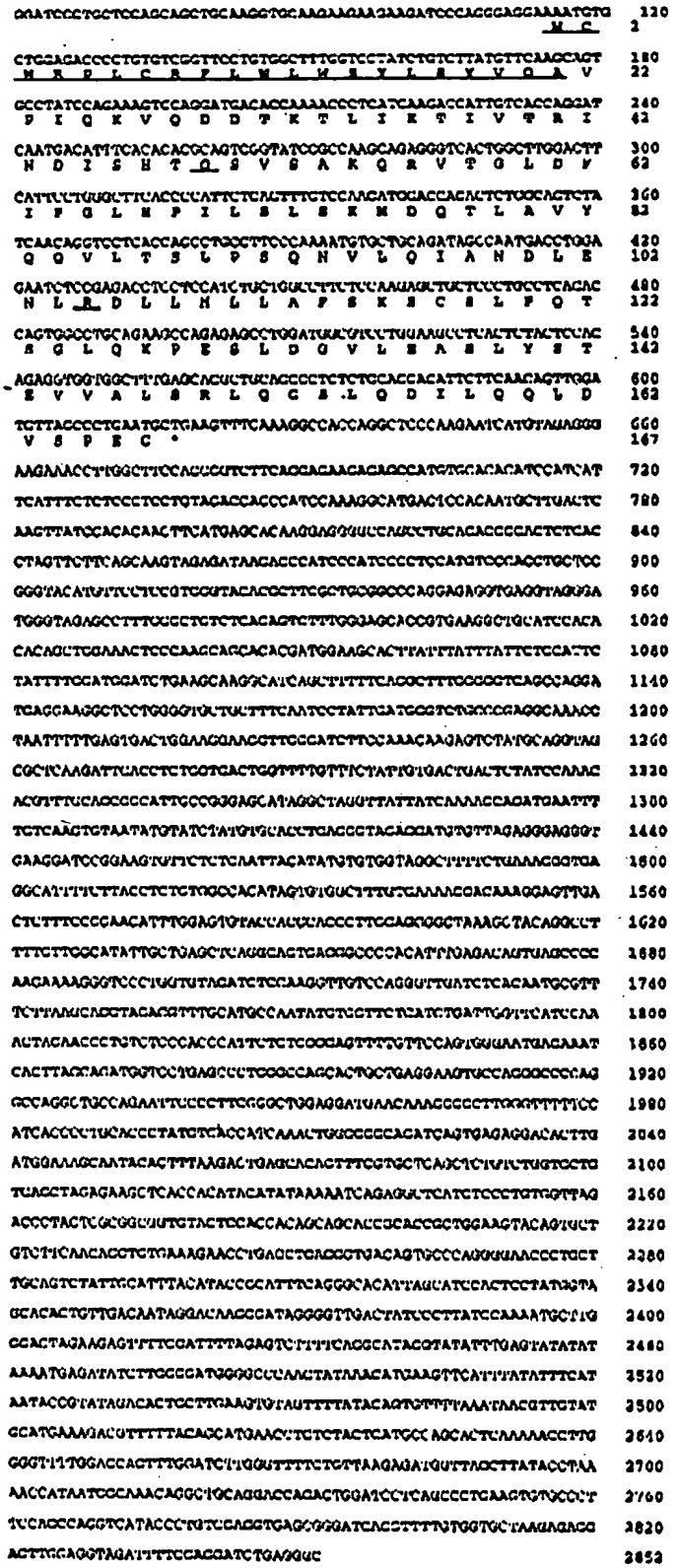


Figure 2

```
---G--GTTG CAAGGCCCAA GAAGCCCA-- -TCCTGGGAA GGAAAATGCA      50
TTGGGGAACC CTGTG-CGGA TTCTTGTGGC TTTGGCCCTA TCTTTTCTAT     100
GTCCAAGCTG TGCCCATCCA AAAAGTCCAA GATGACACCA AAACCCTCAT     150
CAAGACAATT GTCACCAGGA TCAATGACAT TTCACACACG CAGTCAGTCT     200
CCTCCAAACA GAAAGTCACC GGTTTGGACT TCATTCCTGG GCTCCACCCC     250
ATCCTGACCT TATCCAAGAT GGACCAGACA CTGGCAGTCT ACCAACAGAT     300
CCTCACCAGT ATGCCTTCCA GAAACGTGAT CCAAATATCC AACGACCTGG     350
AGAACCTCCG GGATCTTCTT CACGTGCTGG CCTTCTCTAA GAGCTGCCAC     400
TTGCCCTGGG CCAGTGGCCT GGAGACCTTG GACAGCCTGG GGGGTGTCCT     450
GGAAGCTTCA GGCTACTCCA CAGAGGTGGT GGCCCTGAGC AGGCTGCAGG     500
GGTCTCTGCA GGACATGCTG TGGCAGCTGG ACCTCAGCCC TGGGTGCTGA     550
GGCCTTGAAG GTCACTCTTC CTGCAAGGAC T-ACGTTAAG GGAAGGAACT     600
CTGGTTTCCA GGTATCTCCA GGATTGAAGA GCATTGCATG GACACCCCTT     650
ATCCAGGACT CTGTCAATTT CCCTGACTCC TCTAAGCCAC TCTTCCAAAG     700
G                                                         701
```

Figure 3

1	Met His Trp Gly Thr Leu Cys Gly Phe Leu Trp Leu Trp Pro Tyr
16	Leu Phe Tyr Val Gln Ala↓Val Pro Ile Gln Lys Val Gln Asp Asp
31	Thr Lys Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile
46	Ser His Thr Gln Ser Val Ser Ser Lys Gln Lys Val Thr Gly Leu
61	Asp Phe Ile Pro Gly Leu His Pro Ile Leu Thr Leu Ser Lys Met
76	Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile Leu Thr Ser Met Pro
91	Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu Glu Asn Leu Arg
106	Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys His Leu Pro
121	Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly Val Leu
136	Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg Leu
151	Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
166	Gly Cys End

Figure 4

```
Mouse   MCWRPLCRFL WLWSYLSYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS   50
Human   MHWGTLCGFL WLWPYLFYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS

Mouse   VSAKQRVTGL DFIPGLHPIL SLSKMDQTLA VYQQVLTSLP SQNVLQIAND  100
Human   VSSKQKVTGL DFIPGLHPIL TLSKMDQTLA VYQQILTSMP SRNVIQISND

Mouse   LENLRDLLHL LAFSKSCSLP QTSGLQKPES LDGVLEASLY STEVVALSRL  150
Human   LENLRDLLHV LAFSKSCHLP WASGLETLDS LGGVLEASGY STEVVALSRL

Mouse   QGSLQDILQQ LDVSPEC                                     167
Human   QGSLQDMLWQ LDLSPGC
```

Figure 5

1	Met Cys Trp Arg Pro Leu Cys Arg Phe Leu Trp Leu Trp Ser Tyr
16	Leu Ser Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp
31	Thr Lys Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile
46	Ser His Thr Ser Val Ser Ala Lys Gln Arg Val Thr Gly Leu Asp
61	Phe Ile Pro Gly Leu His Pro Ile Leu Ser Leu Ser Lys Met Asp
76	Gln Thr Leu Ala Val Tyr Gln Gln Val Leu Thr Ser Leu Pro Ser
91	Gln Asn Val Leu Gln Ile Ala Asn Asp Leu Glu Asn Leu Arg Asp
106	Leu Leu His Leu Leu Ala Phe Ser Lys Ser Cys Ser Leu Pro Gln
121	Thr Ser Gly Leu Gln Lys Pro Glu Ser Leu Asp Gly Val Leu Glu
136	Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg Leu Gln
151	Gly Ser Leu Gln Asp Ile Leu Gln Gln Leu Asp Val Ser Pro Glu
166	Cys End

Figure 6

1	Met	His	Trp	Gly	Thr	Leu	Cys	Gly	Phe	Leu	Trp	Leu	Trp	Pro	Tyr
16	Leu	Phe	Tyr	Val	Gln	Ala	Val	Pro	Ile	Gln	Lys	Val	Gln	Asp	Asp
31	Thr	Lys	Thr	Leu	Ile	Lys	Thr	Ile	Val	Thr	Arg	Ile	Asn	Asp	Ile
46	Ser	His	Thr	Ser	Val	Ser	Ser	Lys	Gln	Lys	Val	Thr	Gly	Leu	Asp
61	Phe	Ile	Pro	Gly	Leu	His	Pro	Ile	Leu	Thr	Leu	Ser	Lys	Met	Asp
76	Gln	Thr	Leu	Ala	Val	Tyr	Gln	Gln	Ile	Leu	Thr	Ser	Met	Pro	Ser
91	Arg	Asn	Val	Ile	Gln	Ile	Ser	Asn	Asp	Leu	Glu	Asn	Leu	Arg	Asp
106	Leu	Leu	His	Val	Leu	Ala	Phe	Ser	Lys	Ser	Cys	His	Leu	Pro	Trp
121	Ala	Ser	Gly	Leu	Glu	Thr	Leu	Asp	Ser	Leu	Gly	Gly	Val	Leu	Glu
136	Ala	Ser	Gly	Tyr	Ser	Thr	Glu	Val	Val	Ala	Leu	Ser	Arg	Leu	Gln
151	Gly	Ser	Leu	Gln	Asp	Met	Leu	Trp	Gln	Leu	Asp	Leu	Ser	Pro	Gly
166	Cys	End													

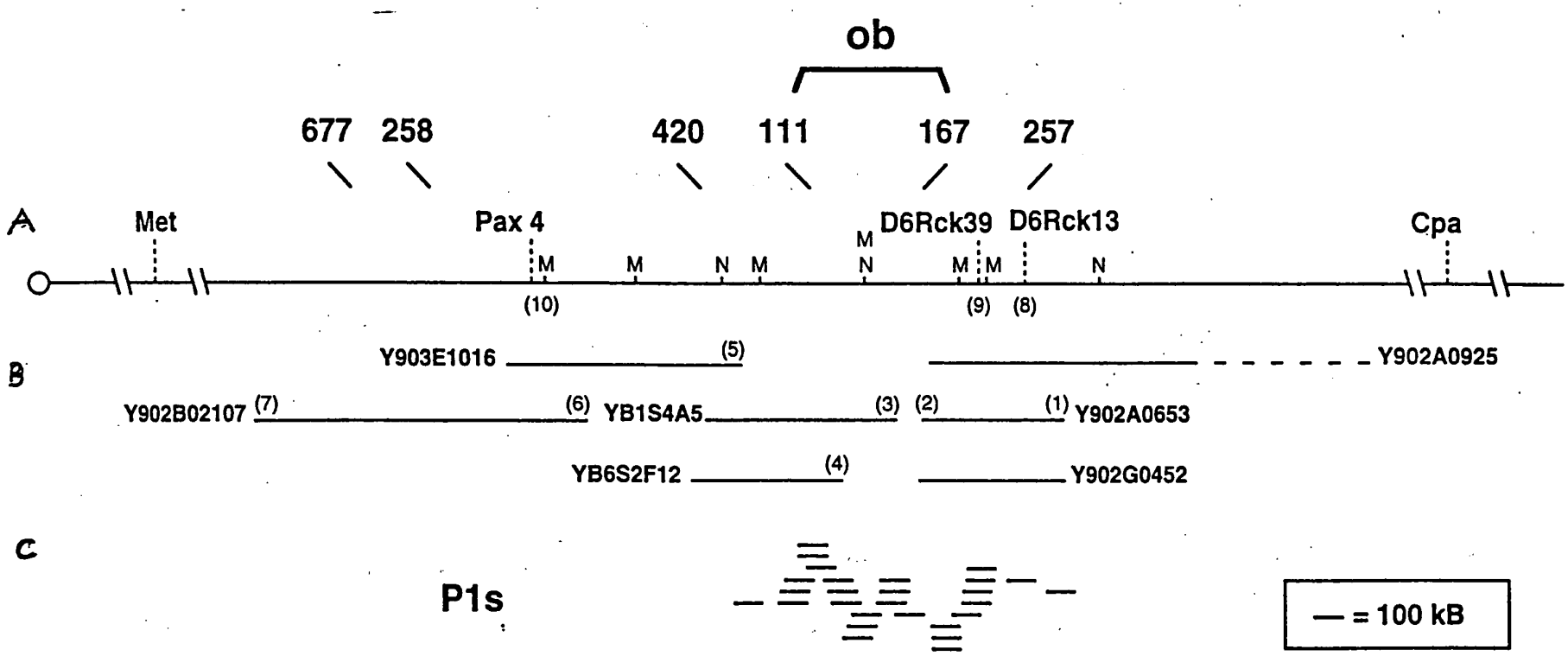


Figure 7

Figure 8

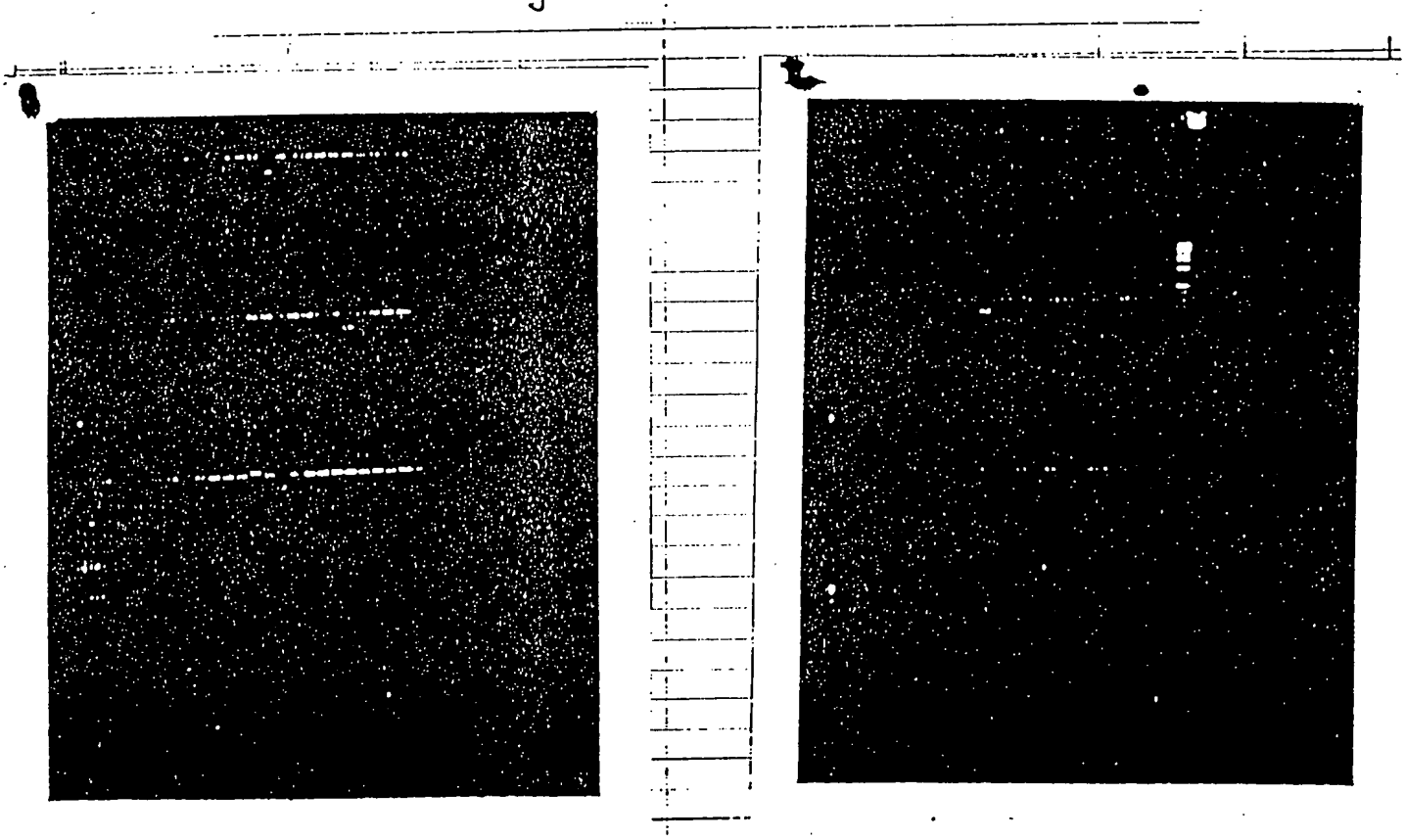


Figure 9

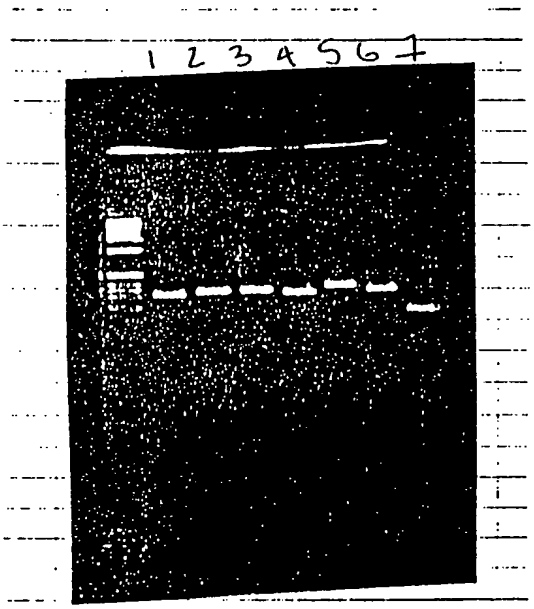


Figure 10

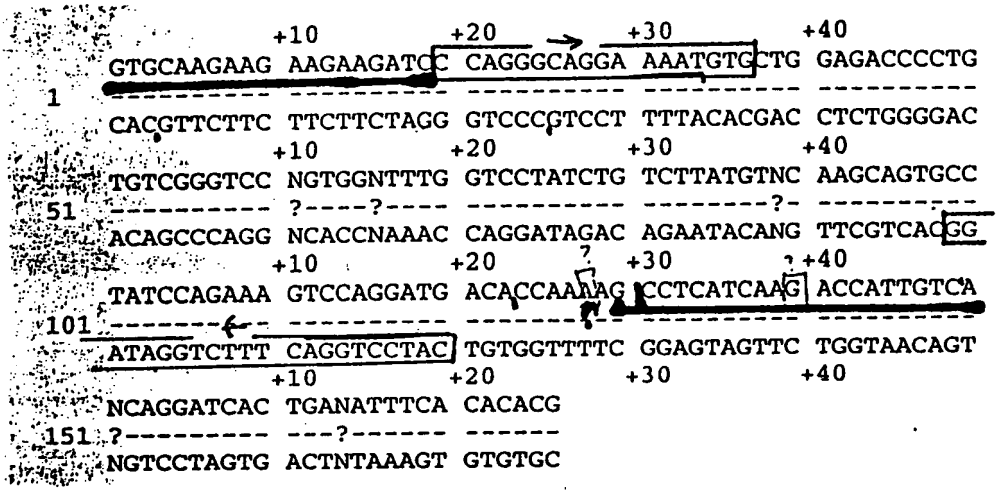

+10 +20 +30 +40
GTGCAAGAAG AAGAAGATCC CAGGGCAGGA AAATGTGCTG GAGACCCCTG
1
CACGTTCTTC TTCTTCTAGG GTCCCGTCCT TTTACACGAC CTCTGGGGAC
+10 +20 +30 +40
TGTCGGGTCC NGTGGNTTTG GTCCTATCTG TCTTATGTNC AAGCAGTGCC
51
ACAGCCCAGG NCACCNAAAC CAGGATAGAC AGAATACANG TTCGTCACGG
+10 +20 +30 +40
TATCCAGAAA GTCCAGGATG ACACCAAAAG CCTCATCAAG ACCATTGTCA
101
ATAGGTCTTT CAGGTCCTAC TGTGGTTTTC GGAGTAGTTC TGGTAACAGT
+10 +20 +30 +40
NCAGGATCAC TGANATTTCA CACACG
151
NGTCCTAGTG ACTNTAAAGT GTGTGC

Figure 11A

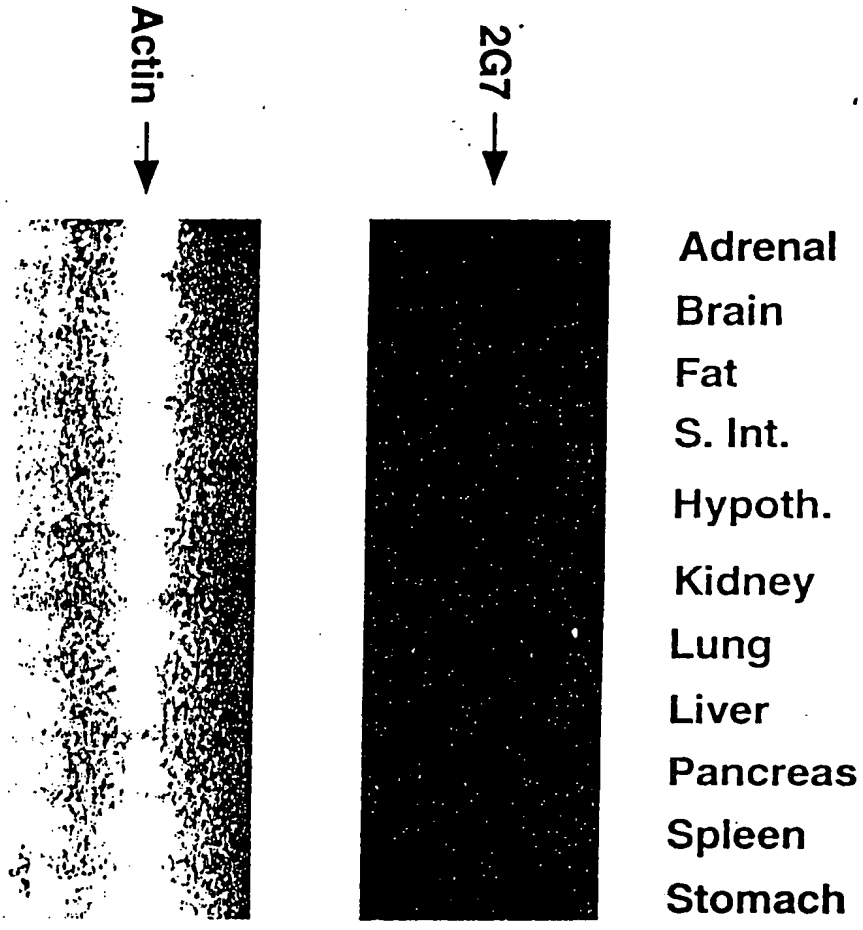

Actin
2G7
Adrenal
Brain
Fat
S. Int.
Hypoth.
Kidney
Lung
Liver
Pancreas
Spleen
Stomach

Figure 11B

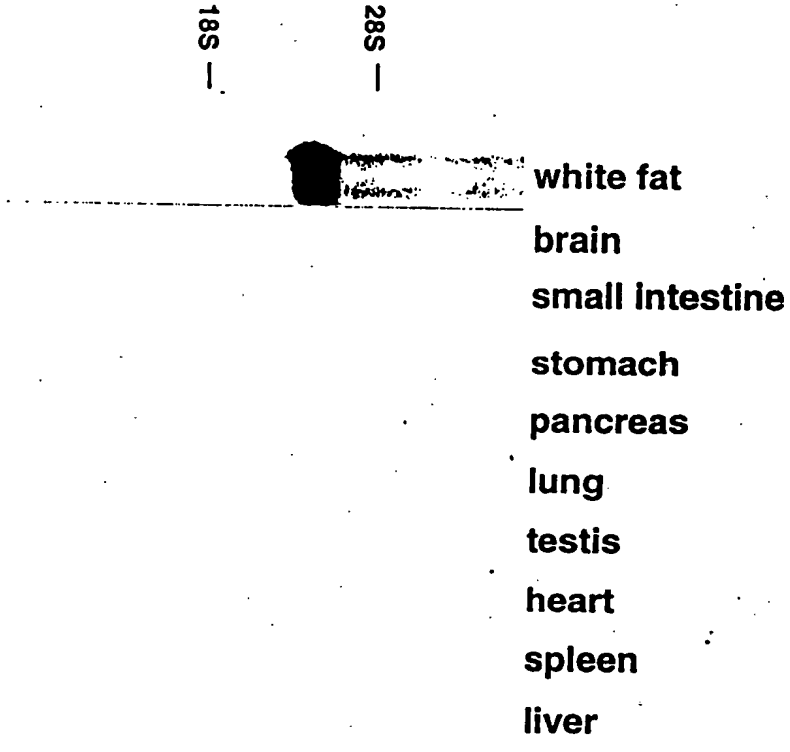

18S
28S
white fat
brain
small intestine
stomach
pancreas
lung
testis
heart
spleen
liver

Figure 12A

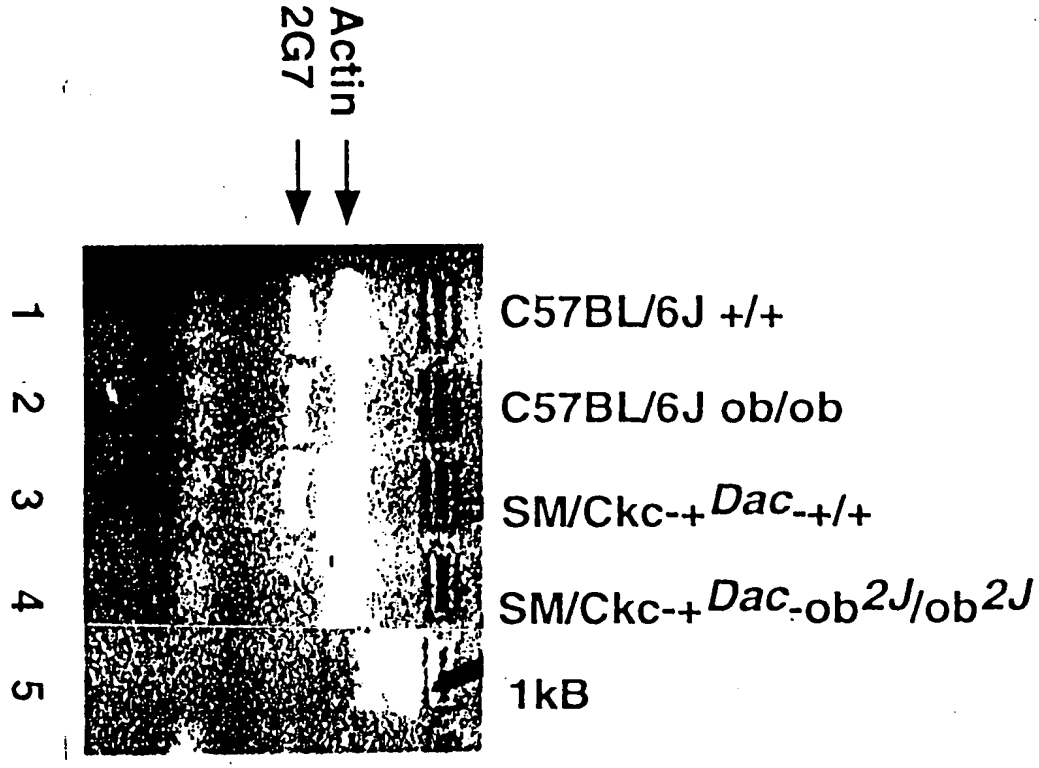

Actin
2G7
1
2
3
4
5
C57BL/6J +/+
C57BL/6J ob/ob
SM/Ckc-+Dac-+/+
SM/Ckc-+Dac-ob2J/ob2J
1kB

Figure 12 B

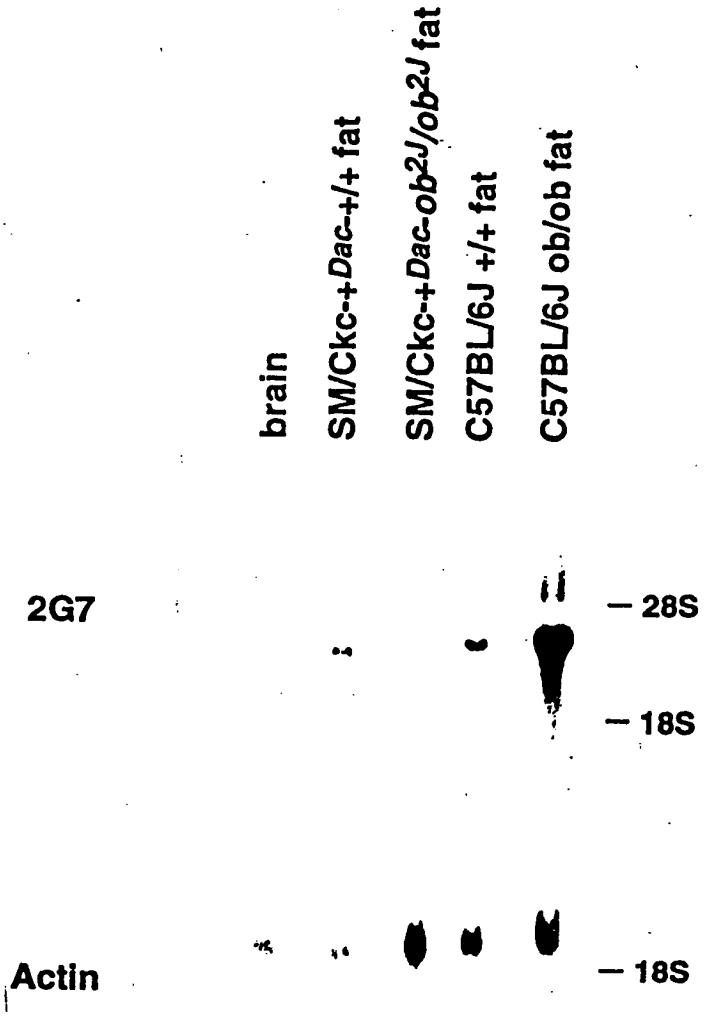


Figure 13

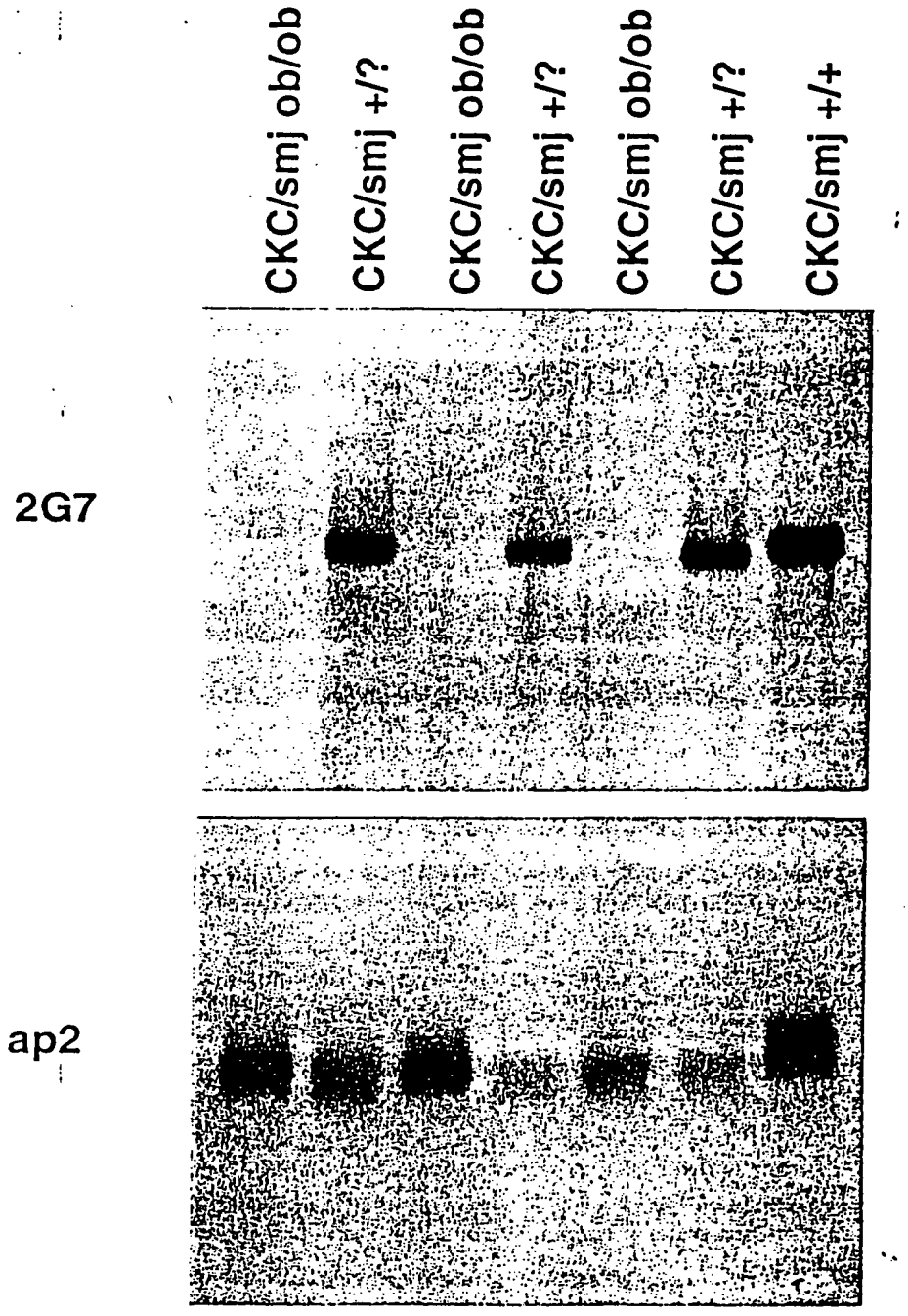

CKC/smj ob/ob
CKC/smj +/?
CKC/smj ob/ob
CKC/smj +/?
CKC/smj ob/ob
CKC/smj +/?
CKC/smj +/+
2G7
ap2

Figure 14

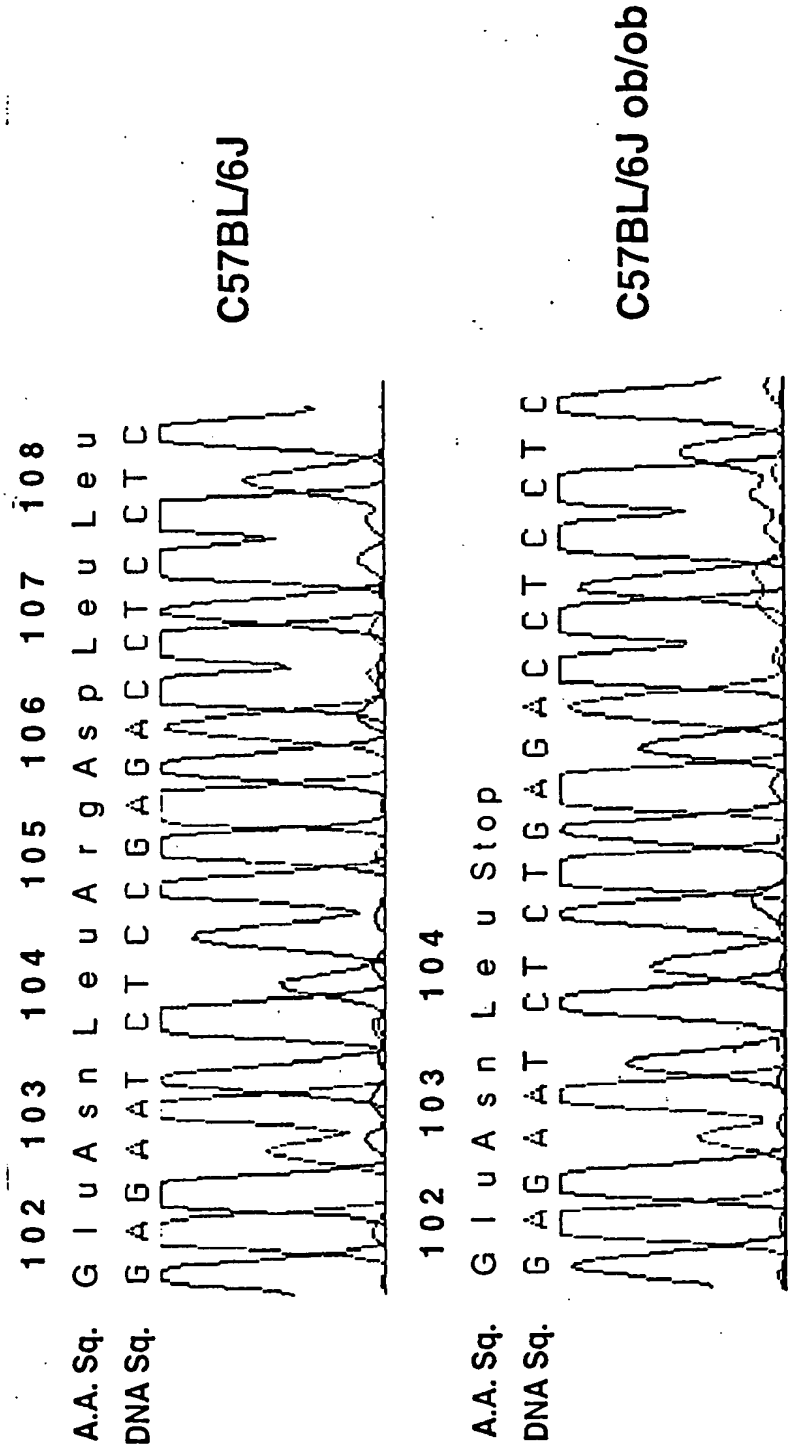

C57BL/6J
A.A. Sq.
DNA Sq.
102 103 104 105 106 107 108
Glu Asn Leu Arg Asp Leu Leu
GAG AAT CTC CGA GAC CTC CTC
C57BL/6J ob/ob
A.A. Sq.
DNA Sq.
102 103 104
Glu Asn Leu Stop
GAG AAT CTC TGA GAC CTC CTC

Figure 15A

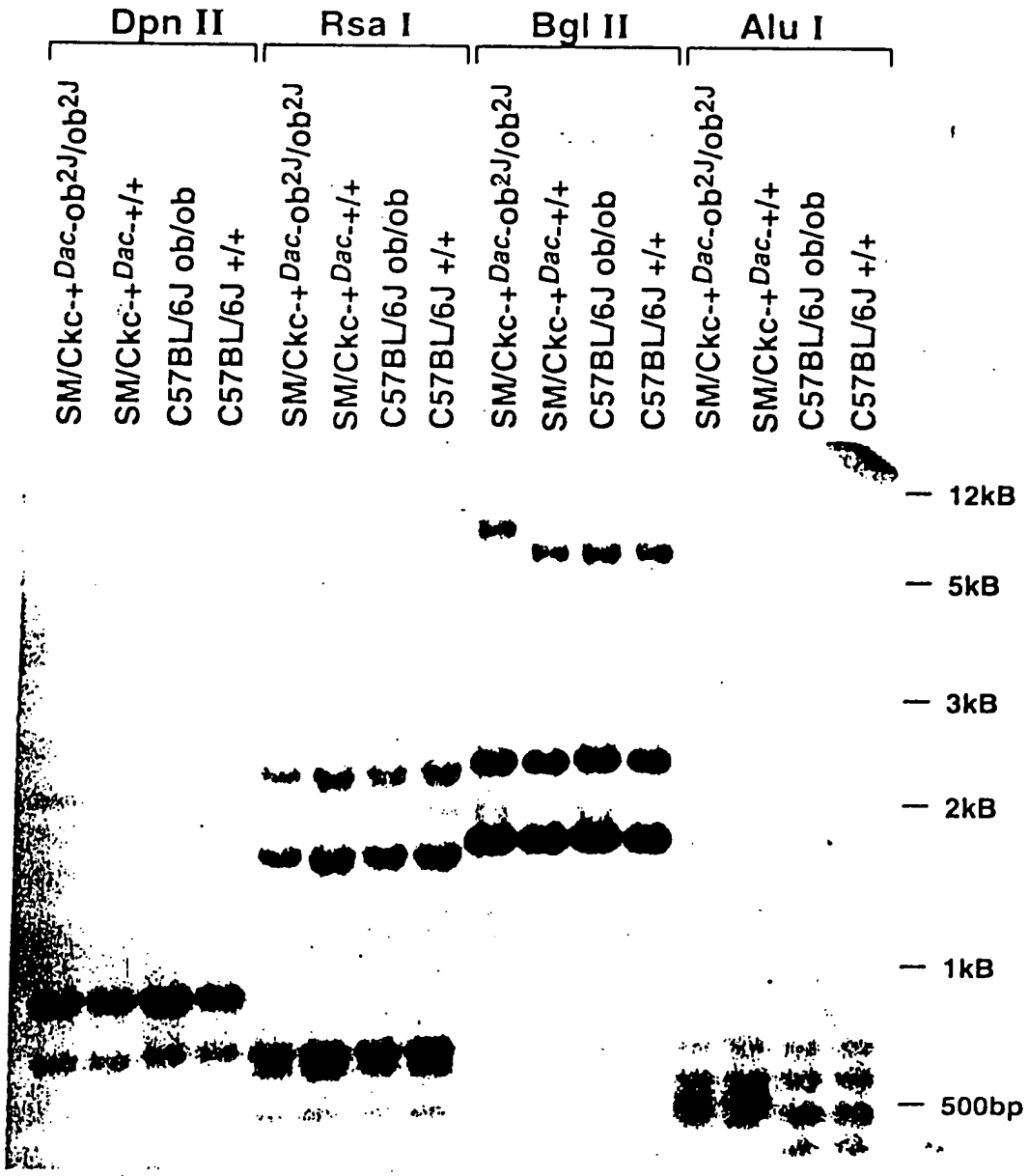


Figure 15B

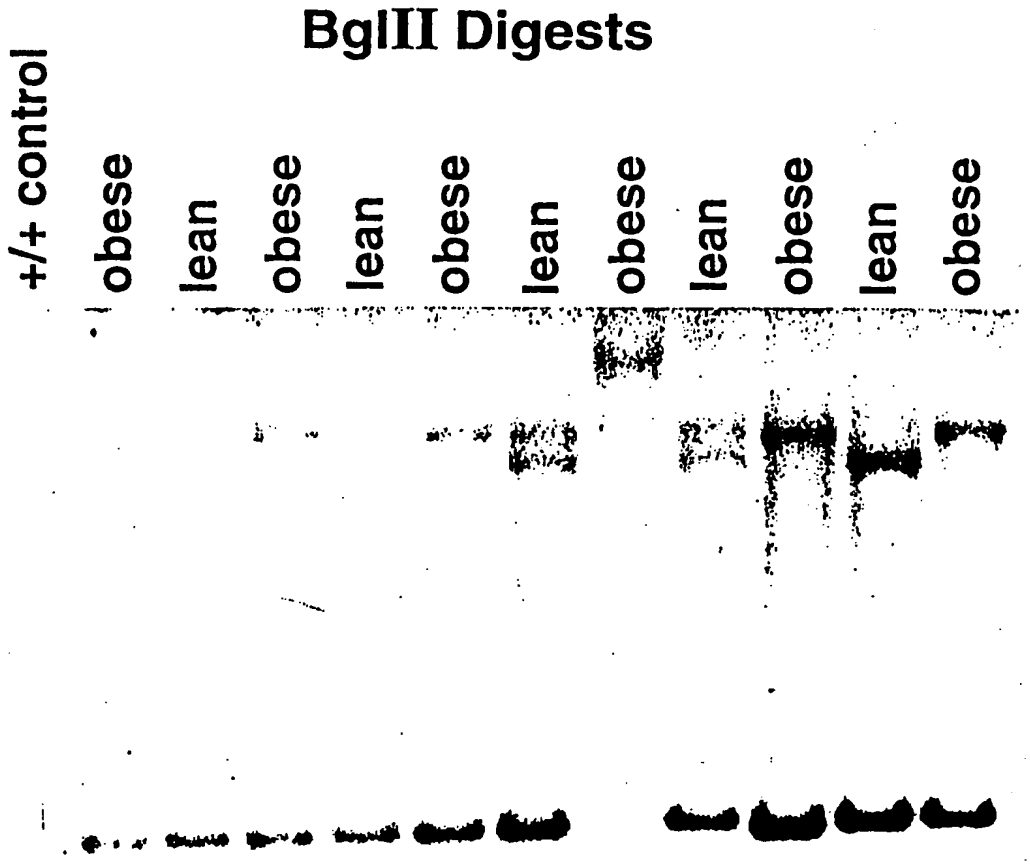
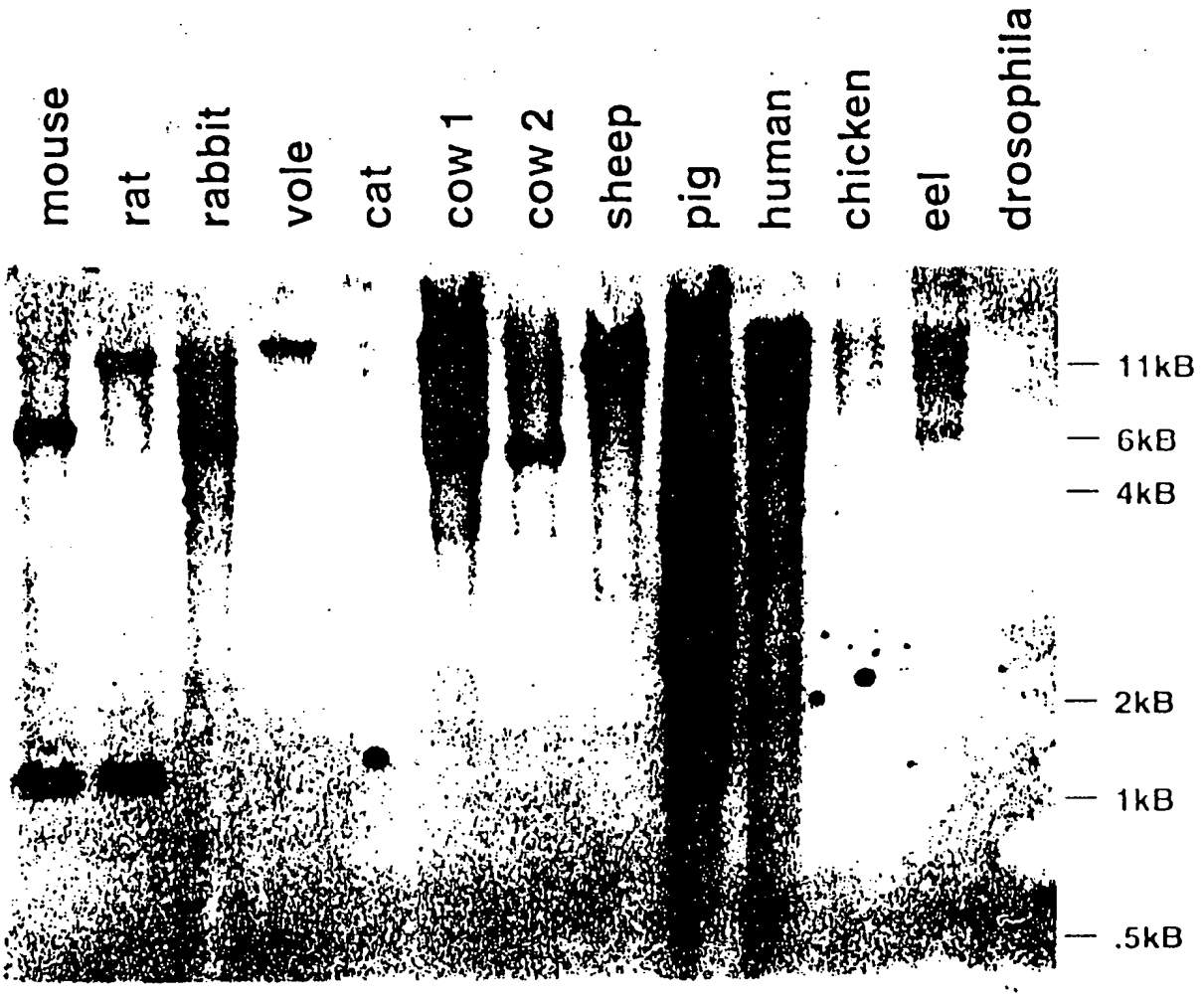

BglII Digests
+/+ control
obese
lean
obese
lean
obese
lean
obese
lean
obese
lean
obese

Figure 16.


mouse
rat
rabbit
vole
cat
cow 1
cow 2
sheep
pig
human
chicken
eel
drosophila
11kB
6kB
4kB
2kB
1kB
.5kB

600-1-081 CLP2 (sheet

08/485943

Figure 17

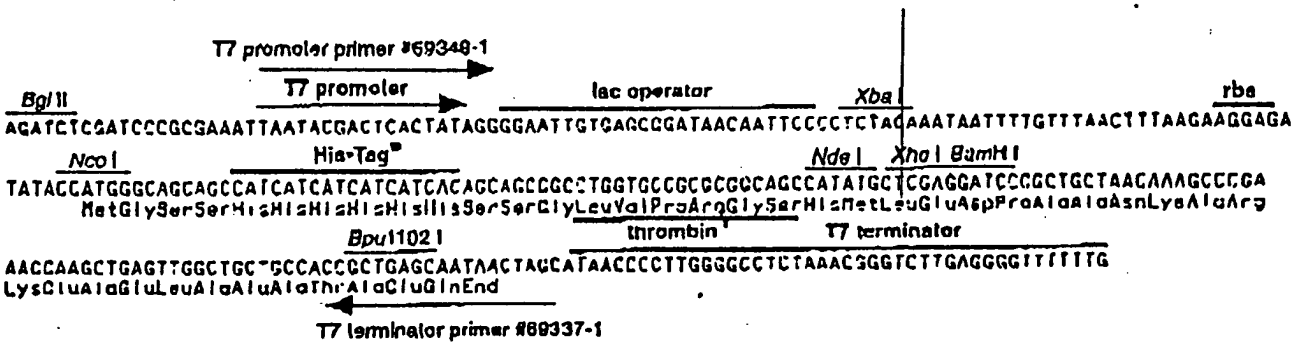


Figure 18A

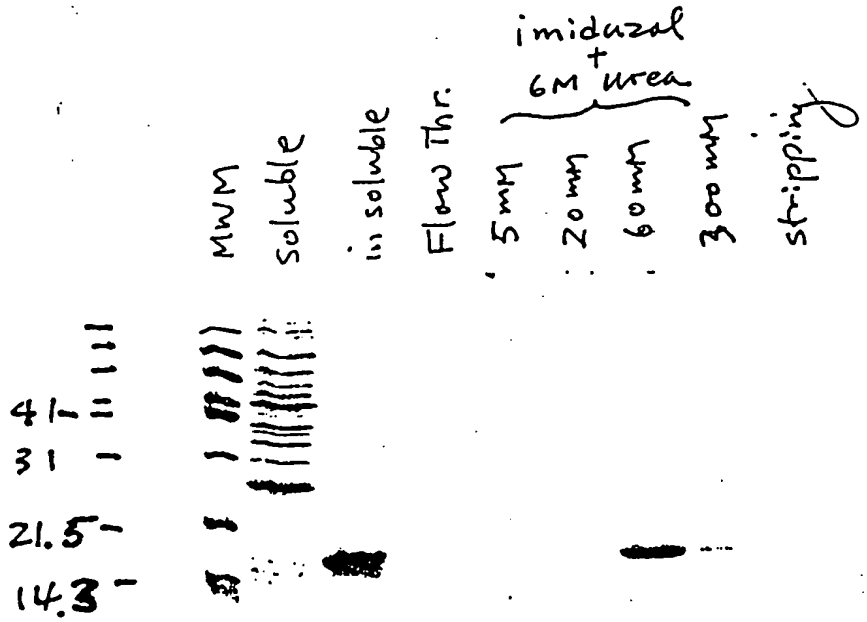


Figure 18B

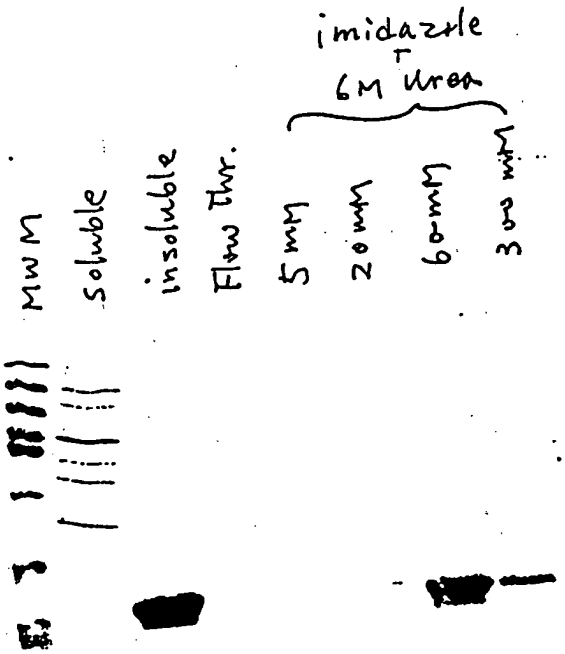

imidazole
6M urea
MWM
soluble
insoluble
Flow thr.
5mM
20mM
60mM
300mM

Figure 19A

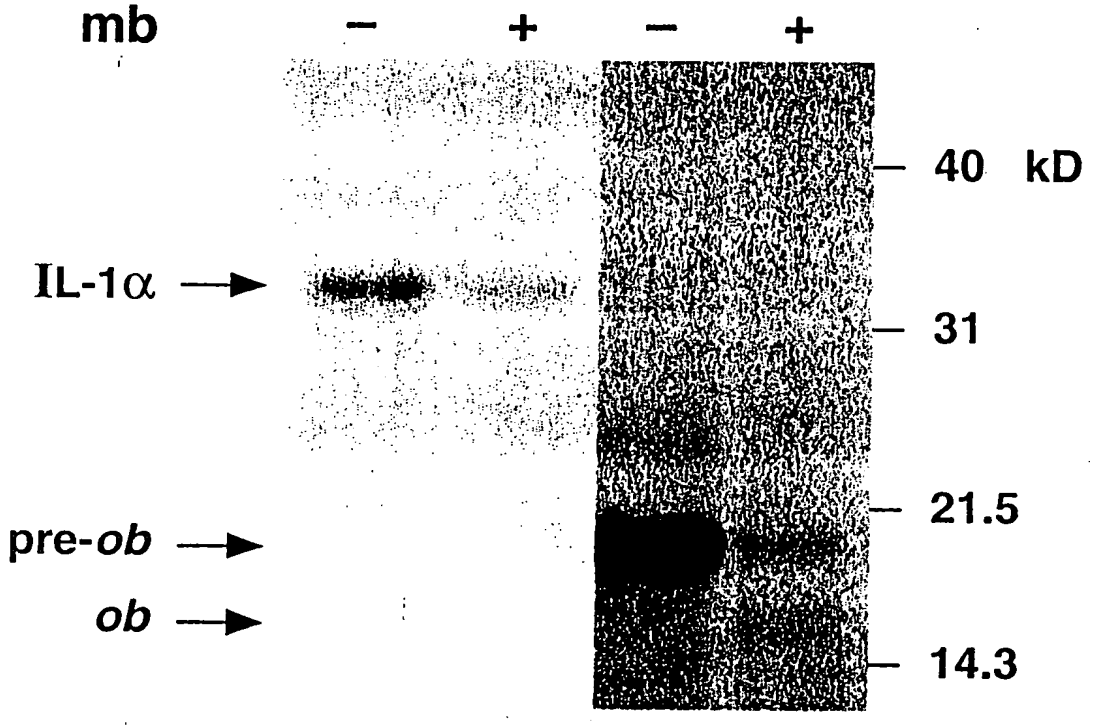


Figure 19B

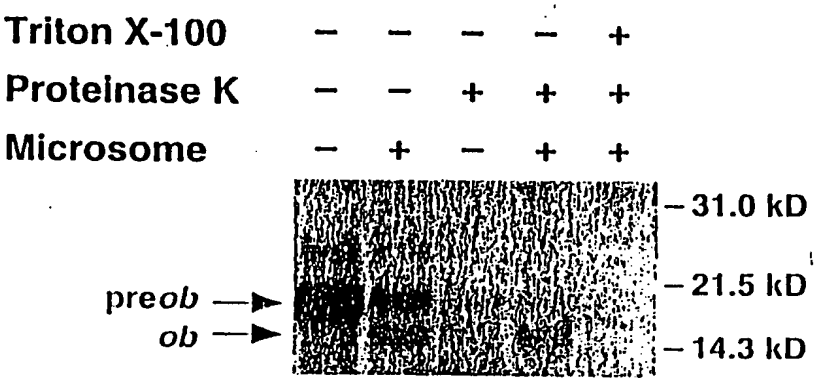

Triton X-100 – – – – +
Proteinase K – – + + +
Microsome – + – + +
– 31.0 kD
– 21.5 kD
– 14.3 kD
preob
ob

Figure 20A

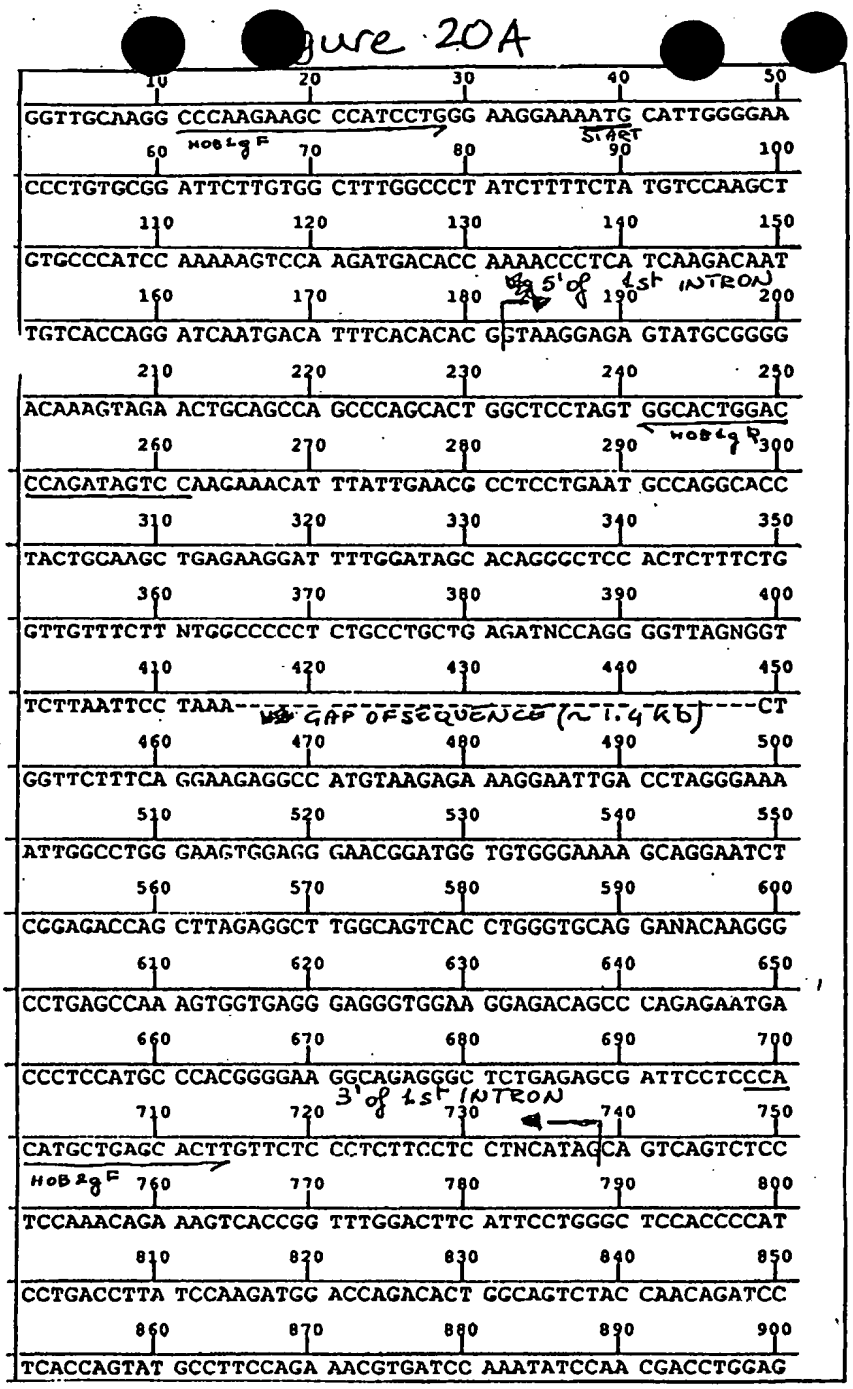

10 20 30 40 50
GGTTGCAAGG CCCAAGAAGC CCATCCTGGG AAGGAAAATG CATTGGGGAA
HOB1gF
START
60 70 80 90 100
CCCTGTGCGG ATTCTTGTGG CTTTGGCCCT ATCTTTTCTA TGTCCAAGCT
110 120 130 140 150
GTGCCCATCC AAAAAGTCCA AGATGACACC AAAACCCTCA TCAAGACAAT
160 170 180 190 200
TGTCACCAGG ATCAATGACA TTTCACACAC GGTAAGGAGA GTATGCGGGG
5' of 1st INTRON
210 220 230 240 250
ACAAAGTAGA ACTGCAGCCA GCCCAGCACT GGCTCCTAGT GGCACTGGAC
HOB1gR
260 270 280 290 300
CCAGATAGTC CAAGAAACAT TTATTGAACG CCTCCTGAAT GCCAGGCACC
310 320 330 340 350
TACTGCAAGC TGAGAAGGAT TTTGGATAGC ACAGGCCTCC ACTCTTTCTG
360 370 380 390 400
GTTGTTTCTT NTGGCCCCCT CTGCCTGCTG AGATNCCAGG GGTTAGNGGT
410 420 430 440 450
TCTTAATTCC TAAA---GAP OF SEQUENCE (~1.4 KB)---CT
460 470 480 490 500
GGTTCTTTCA GGAAGAGGCC ATGTAAGAGA AAGGAATTGA CCTAGGGAAA
510 520 530 540 550
ATTGGCCTGG GAAGTGGAGG GAACGGATGG TGTGGGAAAA GCAGGAATCT
560 570 580 590 600
CGGAGACCAG CTTAGAGGCT TGGCAGTCAC CTGGGTGCAG GANACAAGGG
610 620 630 640 650
CCTGAGCCAA AGTGGTGAGG GAGGGTGGAA GGAGACAGCC CAGAGAATGA
660 670 680 690 700
CCCTCCATGC CCACGGGGAA GGCAGAGGGC TCTGAGAGCG ATTCCTCCCA
3' of 1st INTRON
710 720 730 740 750
CATGCTGAGC ACTTGTTCTC CCTCTTCCTC CTNCATAGCA GTCAGTCTCC
HOB2gF
760 770 780 790 800
TCCAAACAGA AAGTCACCGG TTTGGACTTC ATTCCTGGGC TCCACCCCAT
810 820 830 840 850
CCTGACCTTA TCCAAGATGG ACCAGACACT GGCAGTCTAC CAACAGATCC
860 870 880 890 900
TCACCAGTAT GCCTTCCAGA AACGTGATCC AAATATCCAA CGACCTGGAG
910 920 930 940 950
AACCTCCGGG ATCTTCTTCA CGTGCTGGCC TTCTCTAAGA GCTGCCACTT
960 970 980 990 1000
GCCCTGGGCC AGTGGCCTGG AGACCTTGGA CAGCCTGGGG GGTGTCCTGG
1010 1020 1030 1040 1050
AAGCTTCAGG CTACTCCACA GAGGTGGTGG CCCTGAGCAG GCTGCAGGGG
1060 1070 1080 1090 1100
TCTCTGCAGG ACATGCTGTG GCAGCTGGAC CTCAGCCCTG GGTGCTGAGG
STOP
1110 1120 1130 1140 1150
CCTTGAAGGT CACTCTTCCT GCAAGGACTA CGTTAAGGGA AGGAACTCTG
1160 1170 1180 1190 1200
GCTTCCAGGT ATCTCCAGGA TTGAAGAGCA TTGCATGGAC ACCCCTTATC
HOB2gR
1210 1220 1230 1240 1250
CAGGACTCTG TCAATTTCCC TGACTCCTCT AAGCCACTCT TCCAAAGG

Figure 20B

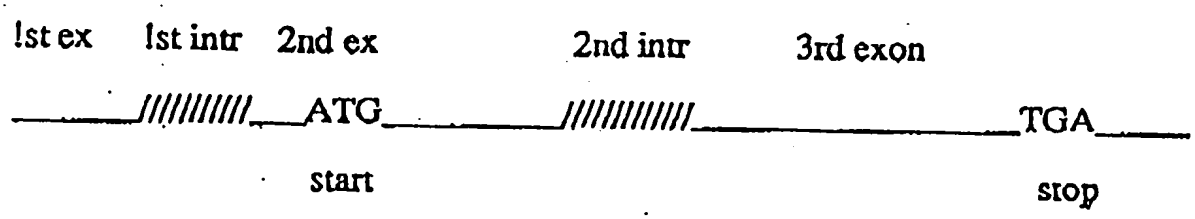


Figure 20c

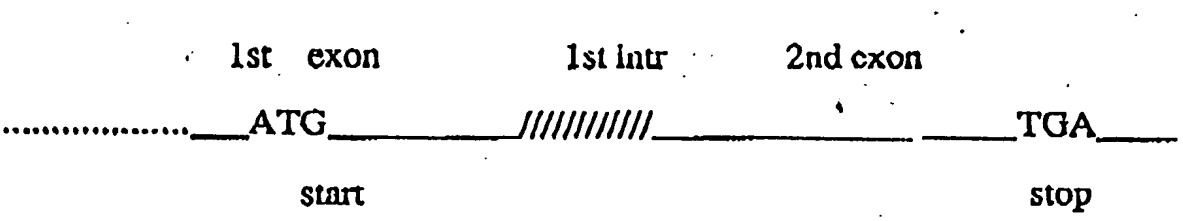


Figure 21A

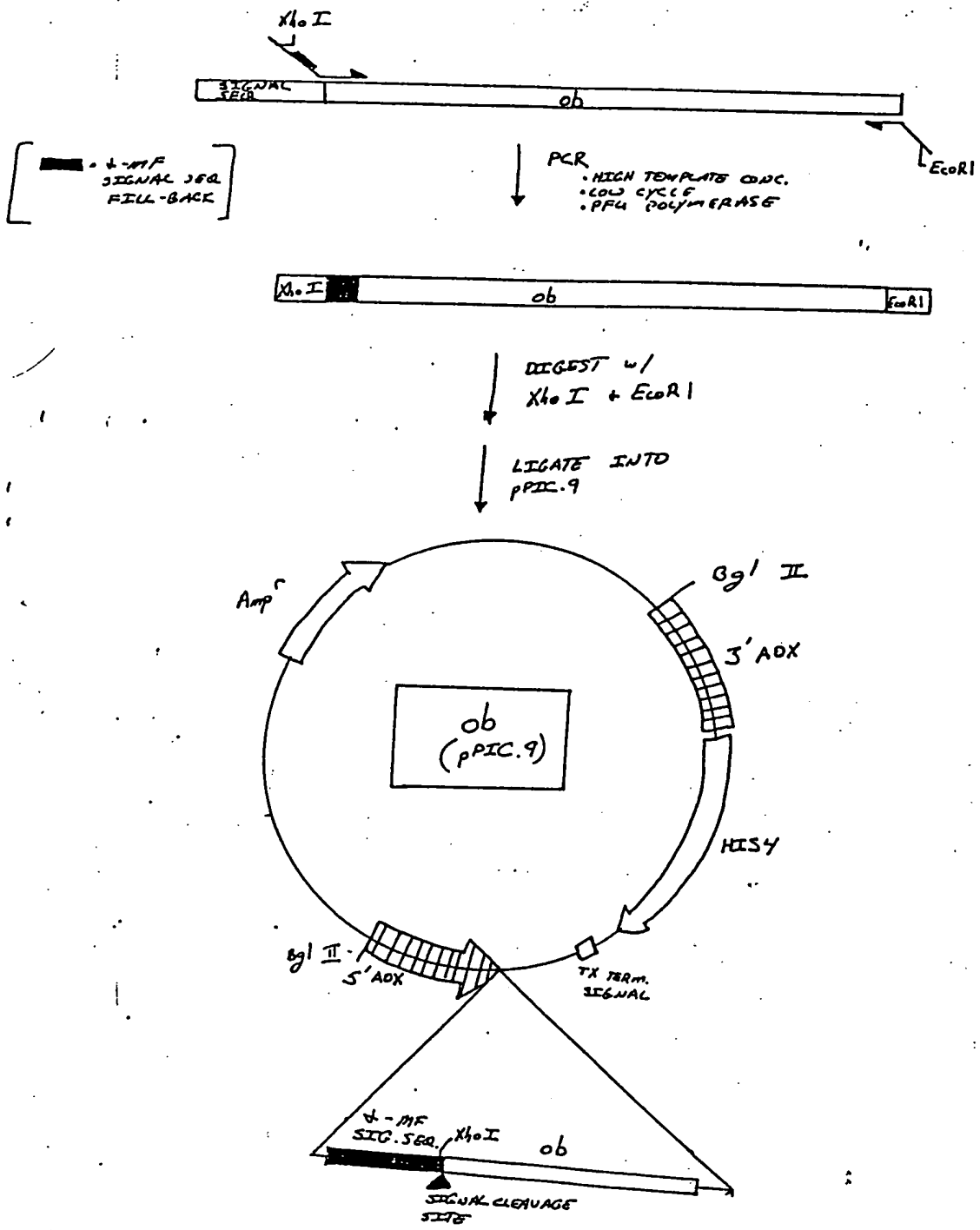

Xho I
SIGNAL SEQ
ob
EcoRI
α-MF SIGNAL SEQ FILL-BACK
PCR
• HIGH TEMPLATE CONC.
• LOW CYCLE
• PFU POLYMERASE
Xho I
ob
EcoRI
DIGEST w/ Xho I + EcoRI
LIGATE INTO pPIC.9
Amp^r
Bgl II
3'AOX
ob (pPIC.9)
HIS4
Bgl II
5'AOX
TX TERM. SIGNAL
α-MF SIG. SEQ.
Xho I
ob
SIGNAL CLEAVAGE SITE

Figure 21 B

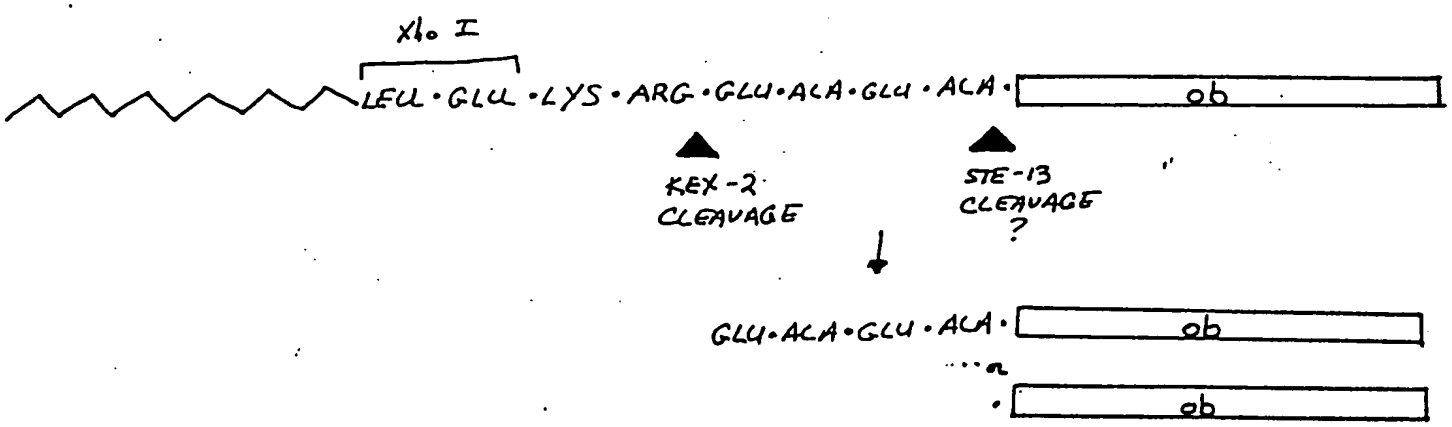


Figure 21 C

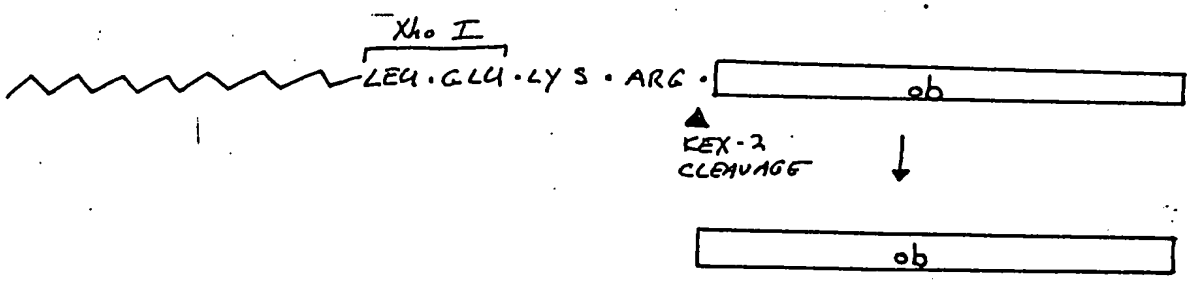


Figure 22A

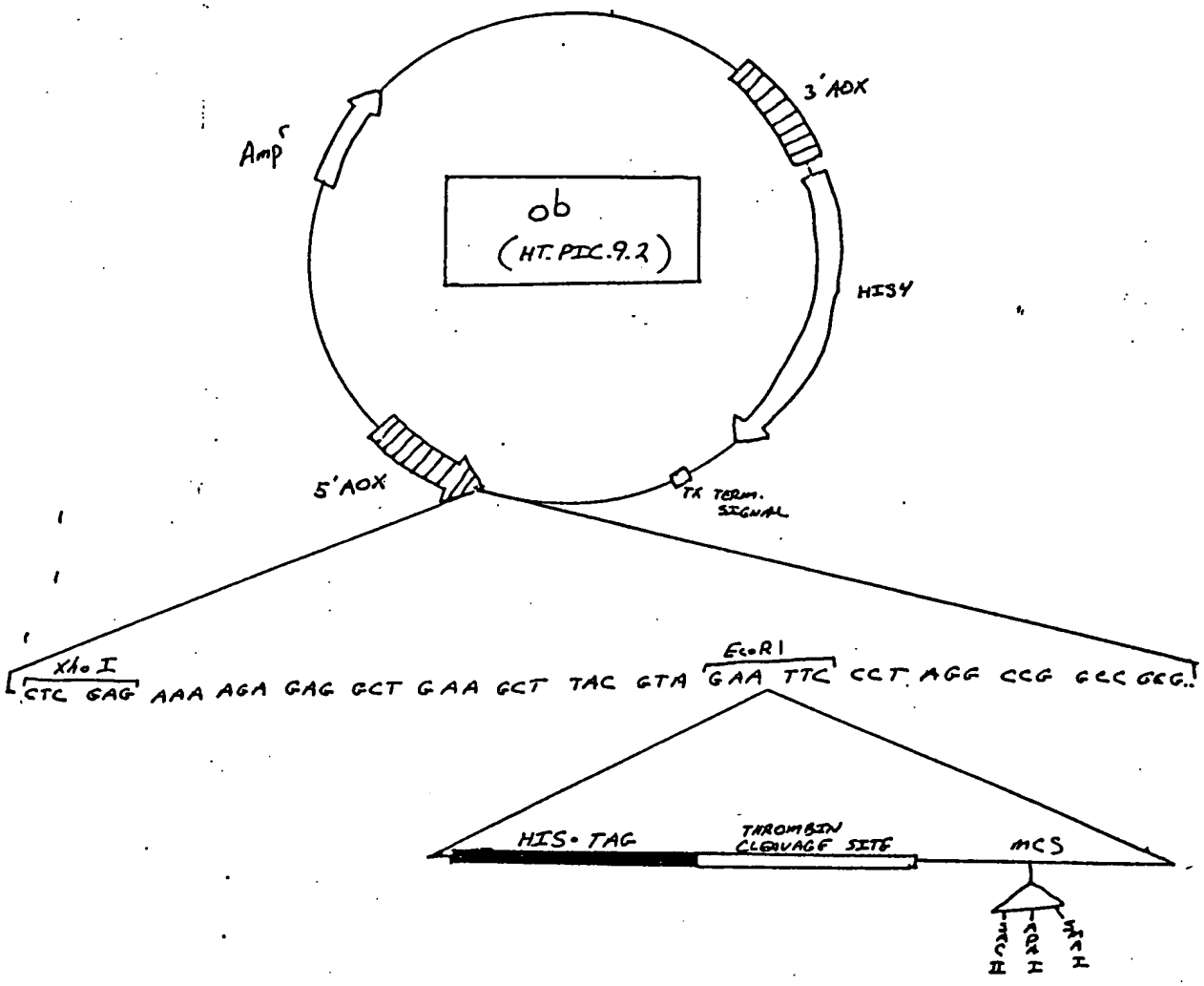


Figure 22B

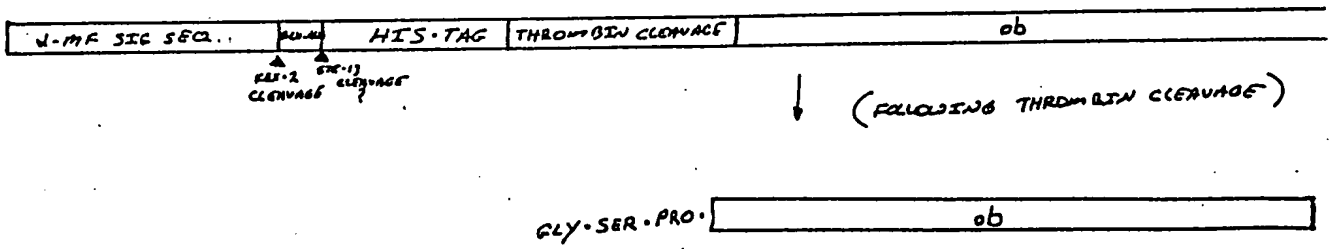

α-MF SIG SEQ..
HIS·TAG
THROMBIN CLEAVAGE
ob
KEX·2 CLEAVAGE
STE·13 CLEAVAGE
?
(FOLLOWING THROMBIN CLEAVAGE)
GLY·SER·PRO·
ob

Figure 23A.

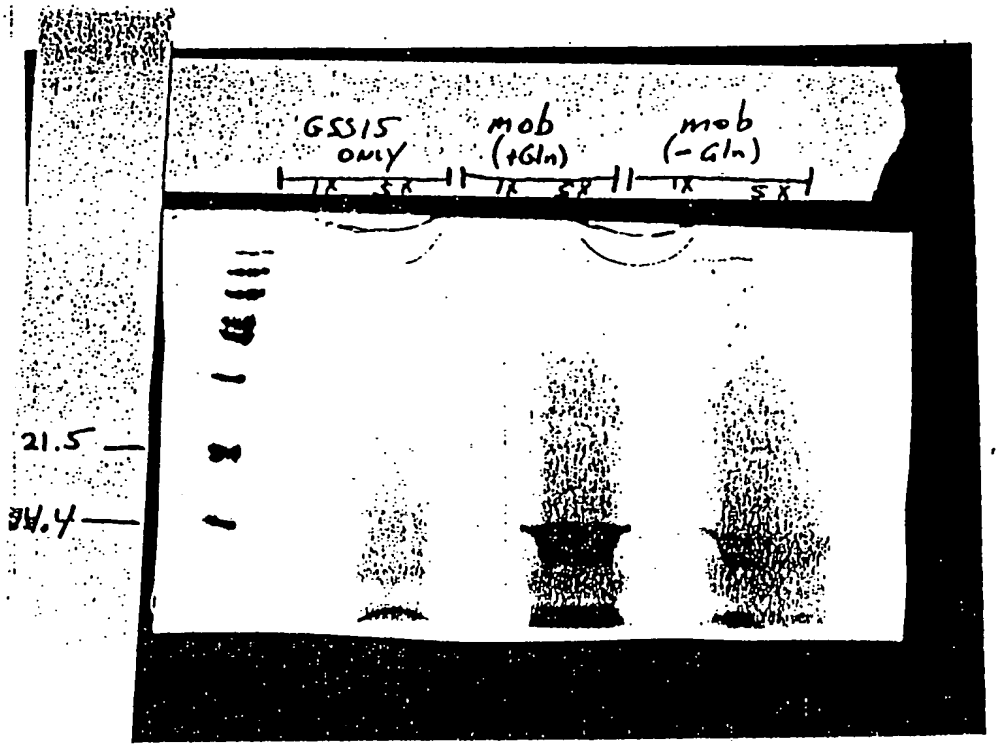


Figure 23B

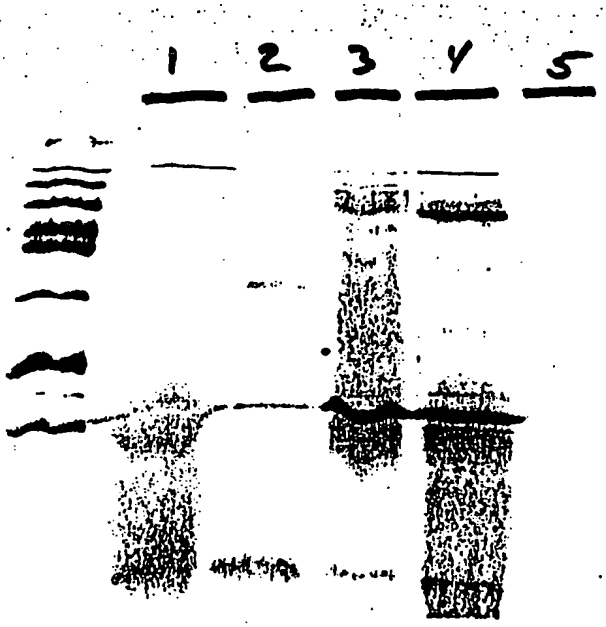


Figure 24A

Figure 24B

Figure 24C

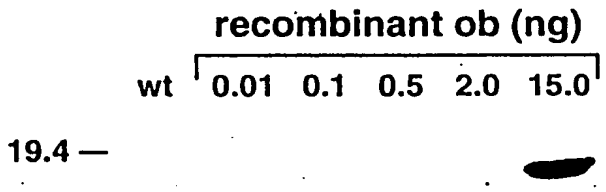


Figure 24 D

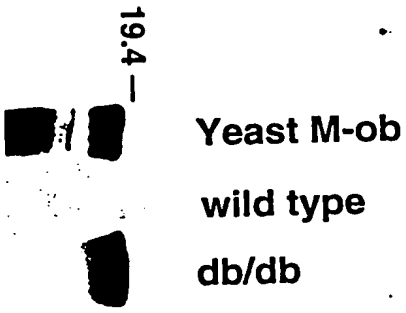


Figure 25A

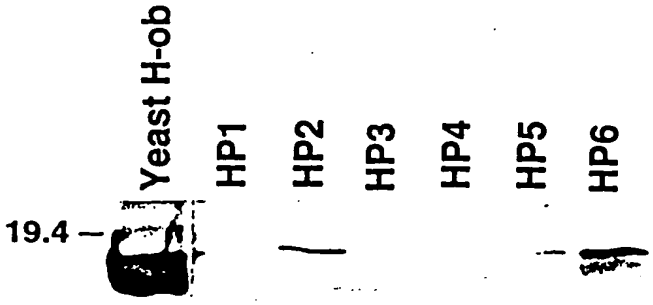


Figure 25 B

ELISA STANDARD CURVE

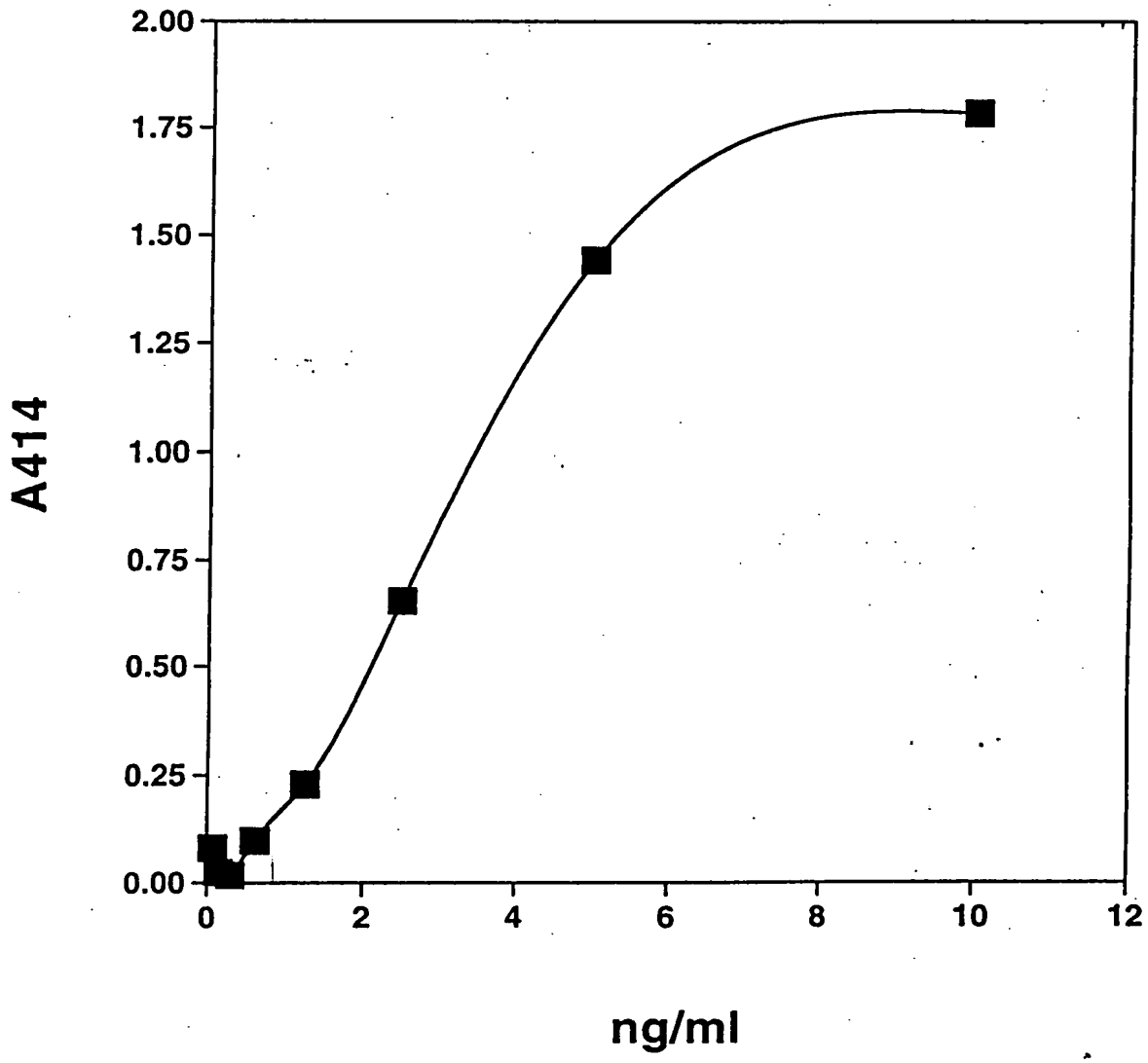


Figure 25C

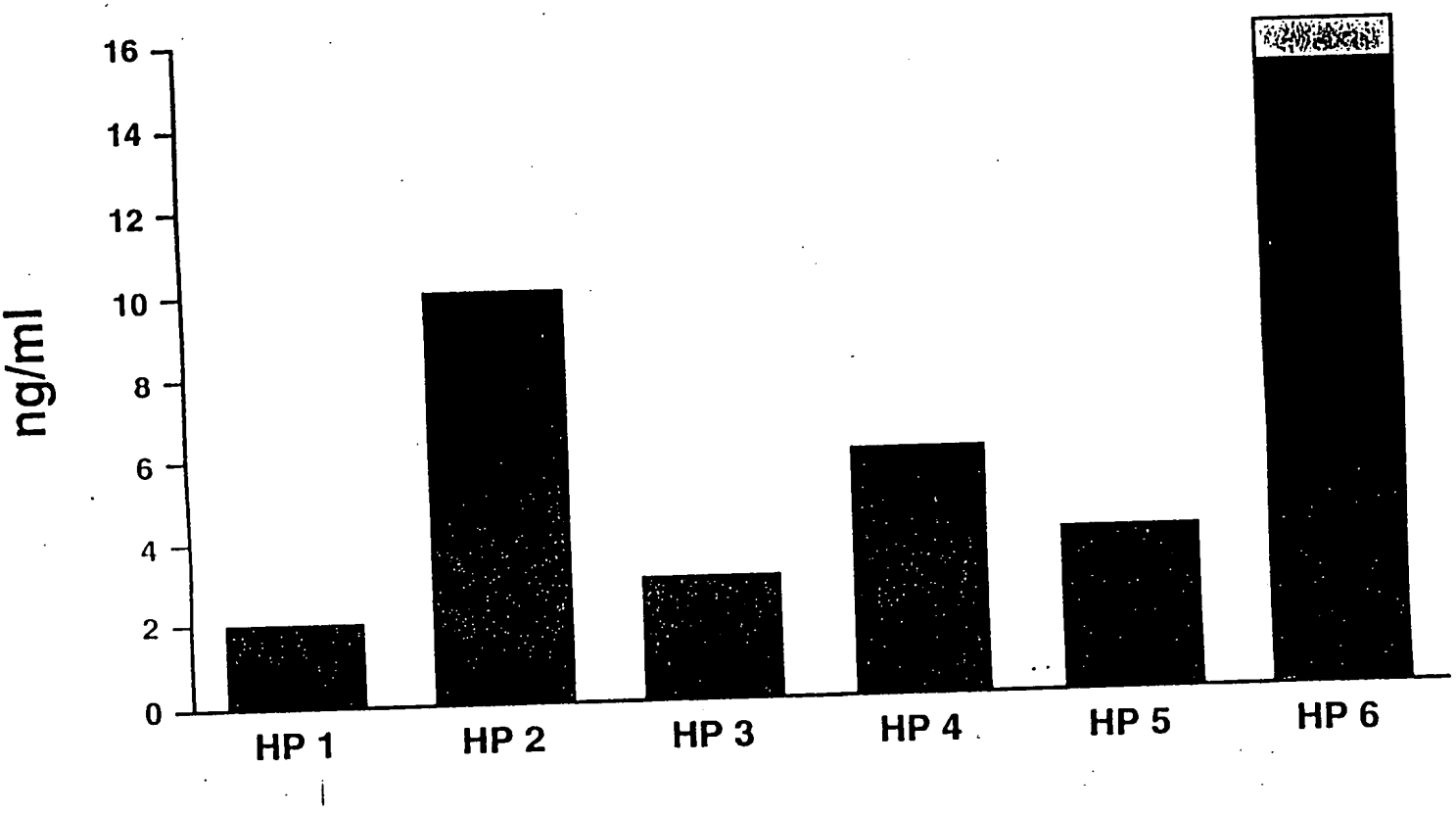


Figure 26A

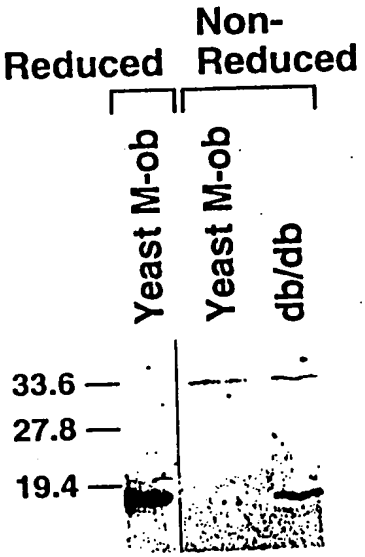
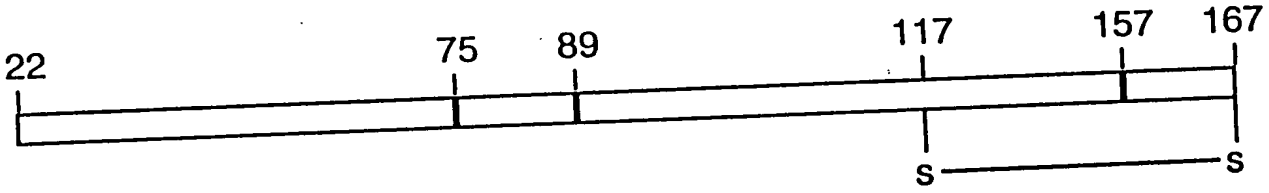


Figure 26 B

Human ob



Peptide	Mass(Da)	
	Expected	Observed
22-167	16,024	16,024 ± 3
22-75	5936.9	5936.6 ± 1
76-89	1562.7	N.D.
90-167	8434.5	8435.6 ± 1
158-167	1131.9	N.D.

Figure 27


Imidazole
marker
soluble
insoluble
FT
4mM
24mM
60mM
300mM
100mM EDTA
41
31
21.5
14.3

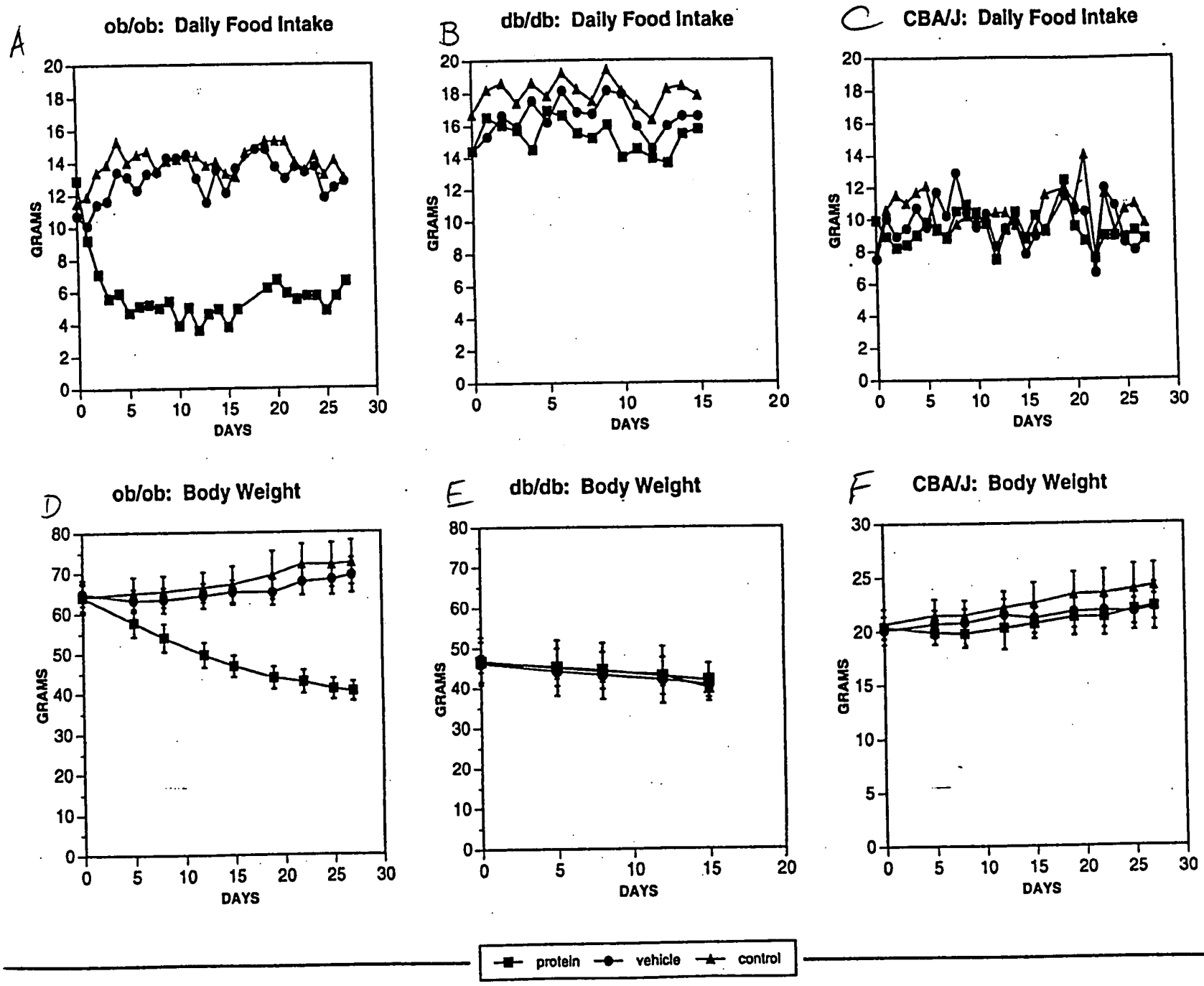


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Figure 28

08/485943

Figure 29A

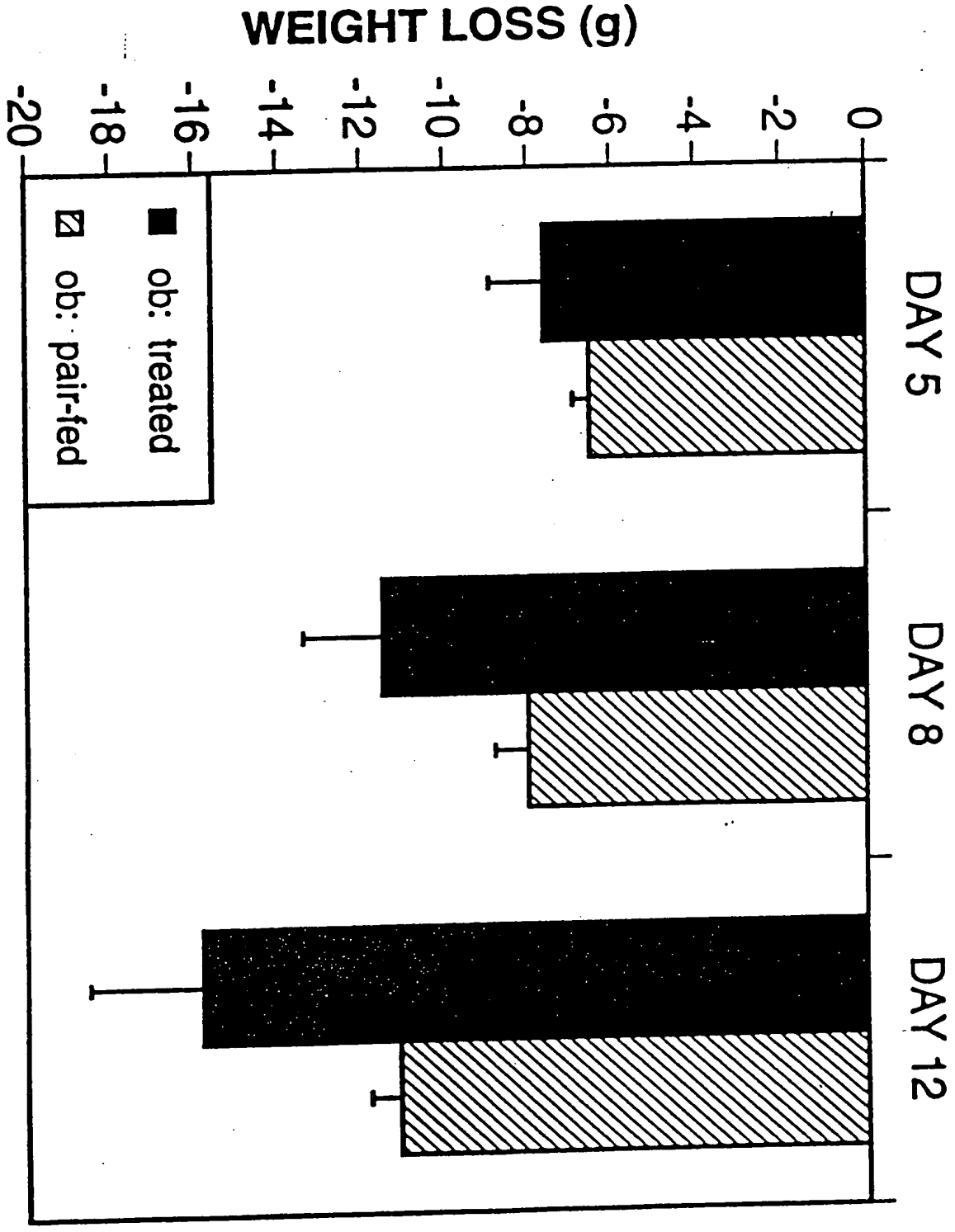
WEIGHT LOSS (g)
-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0
ob: treated
ob: pair-fed
DAY 5
DAY 8
DAY 12

Figure 29B

Figure 29C

Figure 30

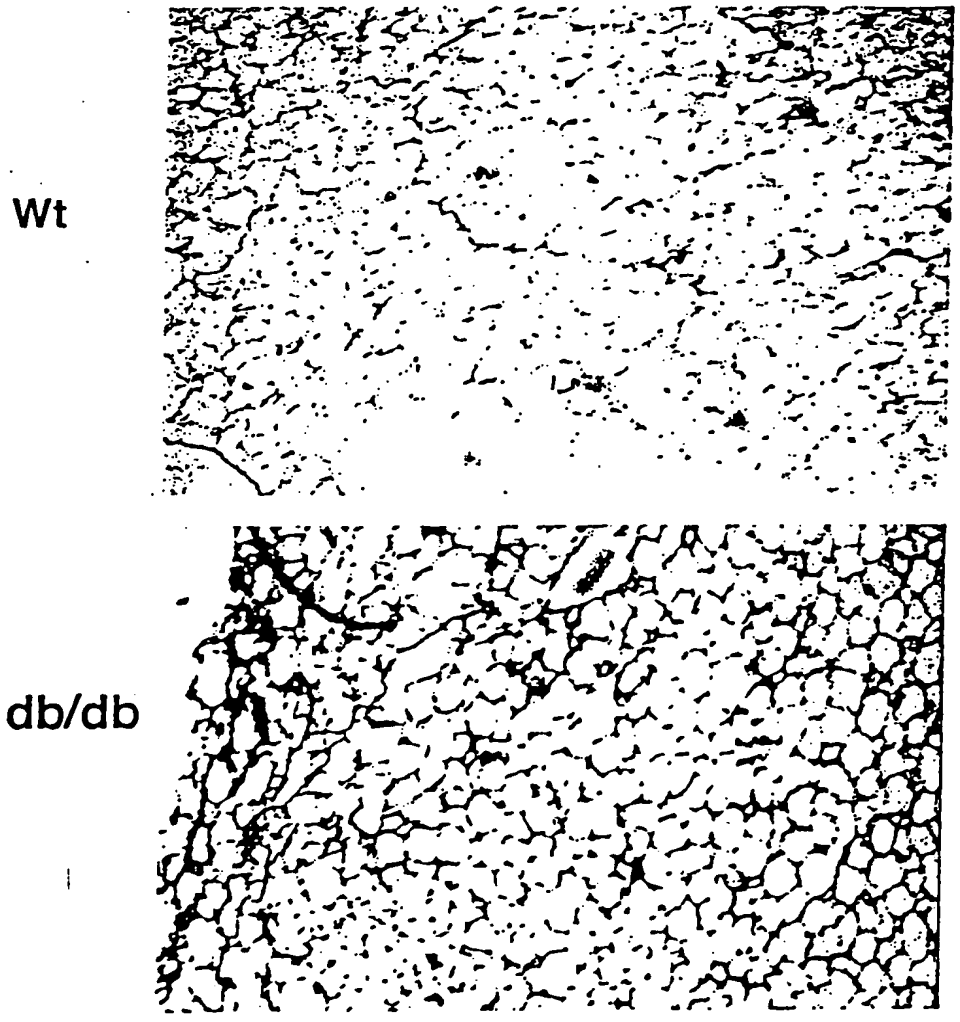

Figure 31

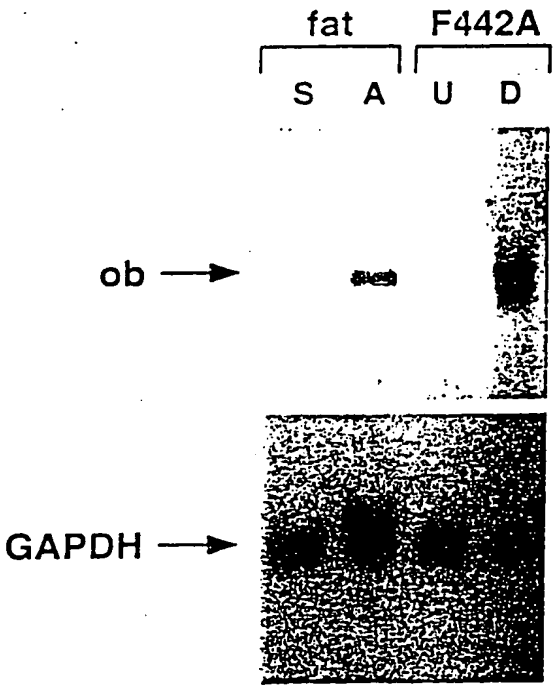

Figure 32A

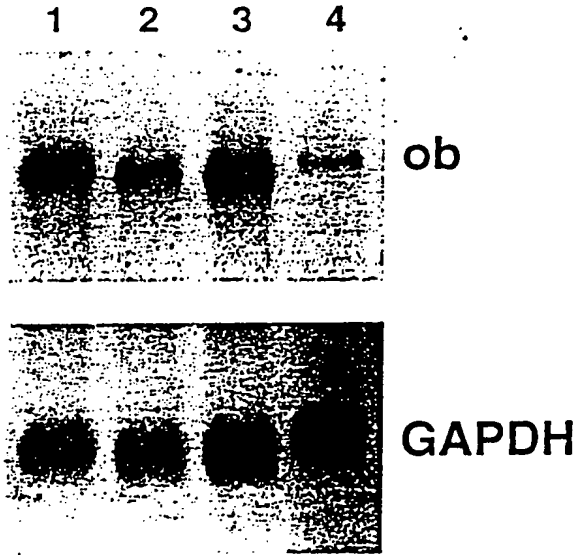

Figure 32 B

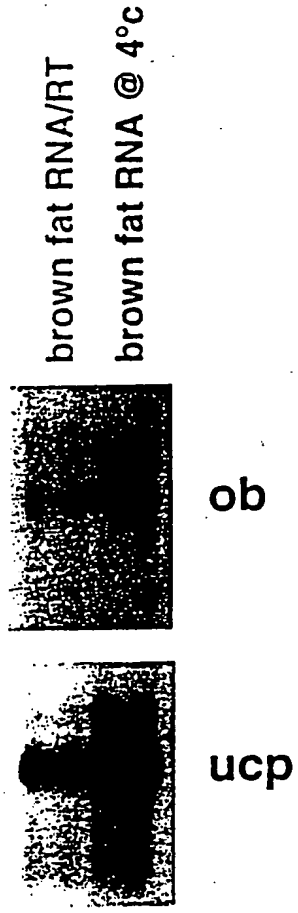

Figure 33

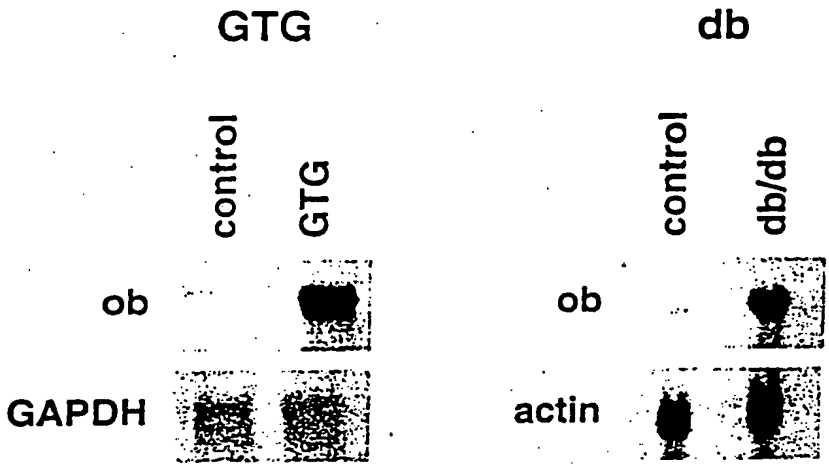

Figure 34

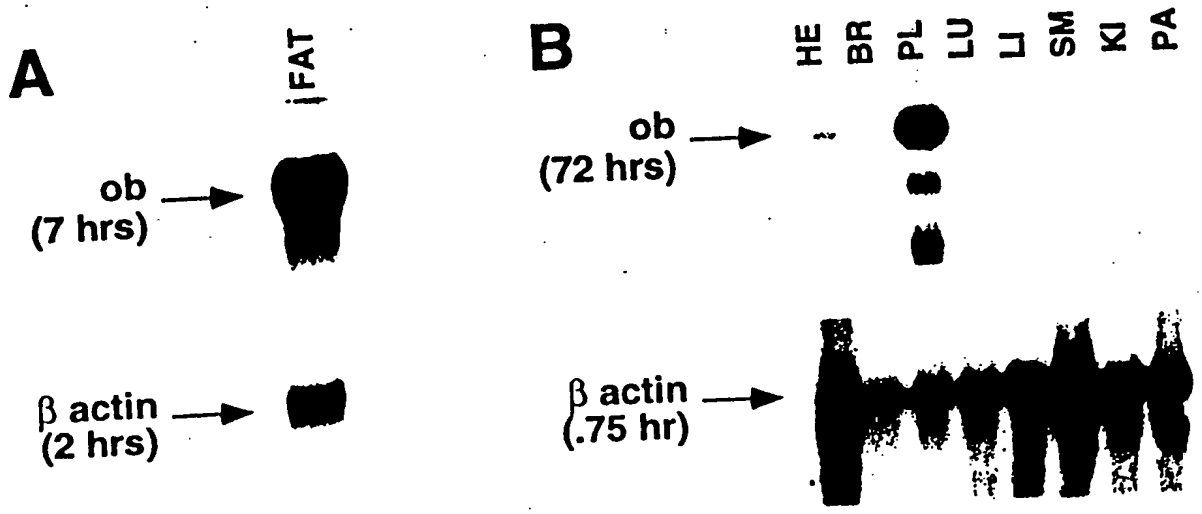

Figure 35